International Journal of Medical Science and Clinical Invention 5(03): 3648-3655, 2018
DOI:10.18535/ijmsci/v5i3.17 **ICV 2016: 77.2**
e-ISSN:2348-991X, p-ISSN: 2454-9576

Research Article

Assessment of Antioxidant Potential and phytochemical screening of Phenolic Compounds of *Gleditsia triacanthos* L Pods

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Abstract:
Objective: The present study aims at the phytochemical screening, the quantification of phenolic compounds of *Gleditsia triacanthos* L pods (Family Leguminosae) and the assessment of their antioxidant potential by in vitro assays.
Subjects and Methods: The pods were extracted with 70% methanol and further partitioned with petroleum ether, chloroform, ethyl acetate, and n-butanol. The residual aqueous fraction has been also recovered. Colorimetric methods using Folin-Ciocalteu reagent, aluminum chloride and Folin-Denis were carried out to estimate total polyphenols, flavonoids and condensed tannins content of extracts. 2,2-diphenyl-1-picrylhydrazyl (DPPH) and total antioxidant capacity (TAC) were used to determine in vitro antioxidant activity.
Results: In vitro phytochemical screening for all the extracts was tested and shown positive result for flavonoids, tannins, cardiac glycosides, sterols and terpenes, saponins and alkaloids. However, all the extracts were free of anthraquinones. The strongest activity against radical scavenging of DPPH was found in the ethyl acetate fraction (IC_{50}= 16,288 ± 0,299 µg/ml) which contains highest amounts of flavonoids (25.160 ± 0.016 mg CE/g), whereas the chloroform fraction showed an important total antioxidant capacity (750,584 ± 129,793 mg AAE/g) with the highest amount of total polyphenols (131.667 ± 2.055 mg GAE/g). When compared to the other fractions, the aqueous fraction presented the lowest antioxidant activity for the two methods.
Conclusion: These data suggest that the pods of *Gleditsia triacanthos* L can be a good natural source of antioxidants that can be beneficial for food and human health.

Key words *Gleditsia triacanthos*, phenolics compound, pods extracts, phytochemical screening, antioxidant activity.

INTRODUCTION

Oxidative stress is defined as an imbalance between the production of free radicals and reactive metabolites, so-called oxidants or ROS (reactive oxygen species), and their elimination by protective mechanisms referred to as antioxidants. This imbalance leads to damage of important biomolecules and cells, with potential impact on the whole organism [1]. The oxidation induced by ROS results in cell membrane disintegration, membrane protein damage, and DNA mutations, which results in aging and further initiates or propagates the development of many diseases such as arteriosclerosis, cancer, diabetes mellitus, liver injury, inflammation, skin damages, coronary heart diseases, and arthritis [2]. Hence antioxidants became a vital part of our lives today since they decreases the adverse effects of reactive species, neutralizes or destroys free radicals such as reactive oxygen and nitrogen species, before they damage cells [3, 4].

The body's antioxidant defense consists of both endogenous and diet-derived exogenous compounds, which can be classified into three broad categories: antioxidant enzymes, chain-breaking antioxidant, and metal binding proteins. These antioxidants are small molecules that can be either water soluble or lipid soluble [5]. As shown in recent years, natural antioxidants discovered in plants have attracted some interest due to their widely acclaimed nutritional and therapeutic values [6]. Antioxidant properties stand to be an essential mechanism of beneficial activity of plant-derived compounds and extracts. Polyphenolic compounds including flavonoids, phenolic acids and tannins have been considered as excellent natural antioxidants [7].

Legumes are considered as nutraceutical food, due to the presence of wide variety of phytochemicals such as phenolic compounds, flavonoids, tannins, and unsaturated fatty acids. The family of fabaceae (Leguminosae) consists of about 770 genera and over 19,500 species world wild and is the third largest angiosperm family in terms of species numbers after Asteraceae and Orchidaceae [8]. The genus *Gleditsia* (Fabaceae)

comprises about 16 species and several amongst them have long been used in traditional medicine. *Gleditsia triacanthos* L., (honey locust) is a deciduous tree belonging to the family Fabaceae. It possesses important biological activities as anti-mutagenic, anticancer, cytotoxic and treating rheumatoid arthritis [9]. The Native Americans used the pods of honey locust as a supplementary food source and medicines were made from various parts of the plant [10].

Therefore, the aim of this study is to investigate phytochemical screening, phenolic contents and the in vitro antioxidant activity of different extracts from *Gleditsia triacanthos* L pods harvested from Norwest of Algeria, which should enhance their therapeutic value and improve the popularization of this specie.

2. MATERIAL AND METHODS

2.1. Plant material

The fruits of *Gleditsia triacanthos.* L were collected during the month of November 2015 from northwest of Algeria (Sidi-Bel-Abbes). Dr. Asma El Zerey-Belaskri researcher at department of environment sciences, university of Sidi-bel-Abbes, confirmed the identity of the plant and a voucher specimen (BVCV, Fab125) was deposited at the herbarium of the laboratory. The fruits were washed with clean water, then completely air-dried in the shade at room temperature. The seeds were removed, and the pods were reduced to powder by electric mill, and kept in closed containers until subjected to extraction process.

2.2. Preparation of extracts

The crude extract was obtained by maceration of 100 g of dried material in Erlenmeyer flask with 500 ml of aqueous methanol (7:3 v/v) for 24 hours. The extraction process was conducted two times to retrieve more of the active compounds. The soaked powder-solvent mixture was filtered through a Whatman No.1 filter paper twice to remove insoluble matrices. The percolated extracts were concentrated with a rotary evaporator (LABOROTA 4000, Heidolph, Germany) at 45°C.

The residue was dissolved in distilled water and was fractionated successively with four increasing solvents polarity, including petroleum ether, chloroform, ethyl acetate, and n-butanol to extract phenolic compounds of *Gleditsia triacanthos.* L pods. The organics fractions and residual aqueous fraction were evaporated at 45°C under reduced pressure. The dried sample of each extract was weighed and the yield was calculated.

2.3. Preliminary phytochemical screening

Preliminary qualitative phytochemical analysis of alkaloids, cardiac glycosides, flavonoids, saponins, Sterols and terpenes, tannins and anthraquinones, was carried out using standard methods according to standard methods[11-14]. After addition of specific reagents to the extracts, the results were analyzed by visual observation of color change or by precipitate formation.

2.4. Determination of phenolic compounds content

2.4.1. Total polyphenol contents

Total phenolic contents were determined in the extracts by Folin - Ciocalteu procedure [15], using gallic acid as a standard. An aliquot (125 µl) of diluted extract, 500 µl of distilled water and 125 µl of Folin-Ciocalteu reagent were mixed in a test tube. It was left at room temperature in order to the reaction take place. After 6 minutes, 1250 µl of 7 % Na_2CO_3 solution was added to the sample solution. The final volume was adjusted to 3 ml with distilled water. The tubes were kept in the dark for 90 minutes at room temperature. Absorbance was measured at 760 nm. Total polyphenol content was expressed as mg of gallic acid equivalents per gram of dry matter (mg GAE g^{-1} DM).

2.4.2. Total flavonoid contents

Total flavonoids were measured by a colorimetric assay according to Dewanto et al. [15]. Briefly, 250 µl of the diluted extract was added to 75 µl of $NaNO_2$ solution (7%). The mixture was incubated for 6 minutes at room temperature. Subsequently, 150 µl of 10% $AlCl_3$ was added to the mixture and was incubated at ambient temperature for an additional 5 min. Following that, 500 µl of NaOH (1M) was added to the mixture. The final volume was adjusted to 2500 µl and carefully mixed. The absorbance was immediately read at 510 nm. Catechin was used as standard and the results are expressed as mg of catechin equivalents per gram of dry matter (mg CE g^{-1} DM).

2.4.3. Condensed tannins

The condensed tannins were determined in extracts by a colorimetric Folin-Denis assay reported by Bajaj and Devsharma [16]. 1 ml of each suitably diluted extract was introduced into test tubes containing 7.5 ml of distilled water. 0.5 ml of Folin-Denis reagent and 1 ml of saturated sodium carbonate solution were then added. The tubes were mixed vigorously and allowed to stand at room temperature for 30 minutes. The absorbance was read at 760 nm against a blank containing distilled water. The amount of total condensed tannins were expressed in milligrams of tannic acid equivalents per gram of dry matter (mg TAE.g^{-1} DM)

2.5. Antioxidant activity

2.5.1. Determination of free radical scavenging activity by DPPH method

The free radical scavenging activity of each extract of the pods of *Gleditsia triacanthos.* L was determined by using a stable (DPPH) according to the procedure described by Sánchez-Moreno [17].

Different concentrations extracts were prepared in test tubes. An aliquot of 50 µL of each concentration was added to 1950 µL of methanol solution of DPPH as free radical source. The mixtures were stirred vigorously and incubated for 30 min in the dark at room temperature. The absorbance of the sample was measured using an UV spectrophotometer at 517 nm

against methanol blank. A negative control was taken after adding DPPH solution. The scavenging activity on the DPPH radical was expressed as inhibition percentage using the following equation:

DPPH scavenging activity (IP %) = ($A_{blank} - A_{sample}/A_{blank}$) X 100

A_{blank}: is the absorbance of the control reaction (containing all reagents except the sample),

A_{sample}: is the absorbance of the test compound.

The ascorbic acid methanol solution was used as positive control.

Calculation of IC_{50} concentration

The extract concentration corresponding to 50% inhibition (IC_{50}) was calculated from the curves plotted of inhibition percentage against extract concentrations.

2.5.2. Total antioxidant capacity (TAC)

The total antioxidant capacity of each extract was determined according to the phosphomolybdenum method as reported by Prieto et al. [18]. The assay is based on the reduction of Mo (VI) to Mo (V) by the sample and formation of a green phosphate Mo (V) complex. Briefly, 0.3 ml of each extract and standard (ascorbic acid) with various concentration were mixed in tubes with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). Tubes were incubated in a boiling water bath at 95 °C minutes. The absorbance was measured at 695 nm against a blank using a spectrophotometer after cooling at room temperature. The blank consisted of all reagents and solvents without the sample. The total antioxidant activity was expressed as micrograms of ascorbic acid equivalents per gram of extract (mg AAE/g) using a calibration curve.

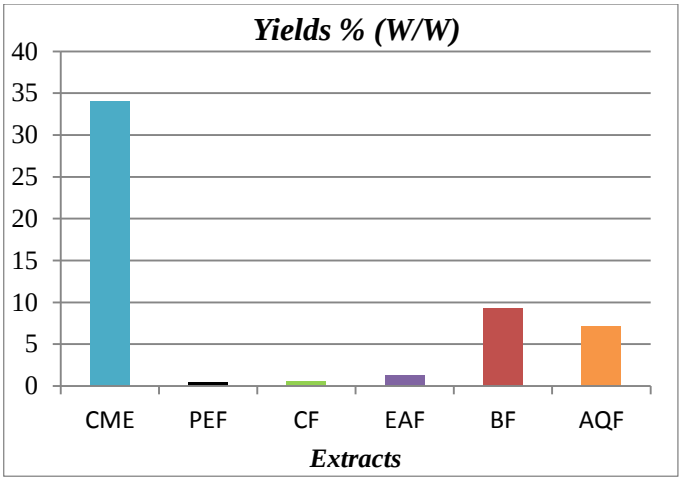
2.6. Statistical analysis

All experiments were performed in triplicate. The results were expressed as mean ± standard deviation. Statistical comparisons were performed with one way analysis of variance, and p values < 0.05 were regarded as significant, using Microsoft Excel 2003 software.

3. RESULTS

3.1. Extraction yields

The extraction yields are shown in Figure 01. The results described in this figure reveal a significant influence ($p < 0.05$) of the extraction capacity of the solvent on the yield.



***CME**: crude methanolic extract, **PEF**: Petroleum Ether Fraction, **CF**: Chloroform Fraction, **EAF**: Ethyl Acetate Fraction, **BF**: n-Butanol Fraction, **AQF:** Aqueous Fraction.*

Figure 01: The yield of different extracts of *Gleditsia Triacanthos* pods

The crude methanolic extract showed the highest extraction yield (34.046 ± 2.133%), and the n-butanol offer the second strongest best extraction efficiency (9.260 ± 2.857 %). In addition, the aqueous fraction exhibited a relatively high extraction yield (7.088 ± 3.621 %). However, compared to the fractions mentioned above, the ethyl acetate fraction revealed lower extraction yield (1.313 ± 0.132 %). Among these fractions, the extraction yields of the chloroform fraction (0.519 ± 0.259 %) and petroleum ether fraction (0.435 ± 0.149 %) were the lowest.

3.2. Preliminary phytochemical screening

The phytochemical characteristics of *Gleditsia Triacanthos* L pods extracts investigated are summarized in Table 1. Phytochemical screening had highlighted the wealth of pods in various classes of secondary metabolites. The results provide evidence of the presence of flavonoids, tannins, cardiac glycosides, sterols and terpenes, saponins and alkaloids depending on the solvents used. However, the results revealed the absence of anthraquinones in all solvents. Three groups of bioactive compounds are present in all extracts; tannins, alkaloids and cardiotonic glycosides. Sterols and terpenes are present in the crude methanolic extracts, and the n-butanol fraction, and were not detected in the other fractions. Saponins and flavonoids were absent in petroleum ether fraction.

Table 1: Phytochemical screening of phenolic extracts of *Gleditsia Triacanthos*. L pods.

	Extracts						
Compounds	***Tests***	***CME***	***PEF***	***CF***	***EAF***	***BF***	***AQF***
Flavonoids	Cyanidin test	++	-	+	+++	+++	++
Tannins	Ferric chloride test	++	+	++	+++	+++	++
Cardiac glycosides	Killer killiani test	+	+	+	+	+	+

Sterols and terpenes	Liebermann test	+	-	-	-	++	-
Saponins	Foam test	+	-	+	+++	++	+++
Anthraquinones	Borntraëger test	-	-	-	-	-	-
Alkaloids	Dragendorff test	++	+	+	+	+	+
	Bouchardat test	++	+	+	+	+	+
	Mayer and Wagner test	++	+	+	+	+	+

(+) = present; (-) = absent

3.3. Determination of phenolic compounds content

The results of the determination of total polyphenols, flavonoids and condensed tannins in the *Gleditsia triacanthos* pods extracts shown in Table 2, revealed the presence of these compounds in the pods of the plant studied with different amounts depending on the extraction solvent.

3.3.1. Total polyphenols

The value of total phenol content varied, and ranged from 23.833 ± 3.171 to 131.667 ± 2.055 mg GAE/g. Chloroform fraction was found to contain the highest amount of phenolic compounds (131.667 ± 2.055 mg GAE/g) followed by ethyl acetate fraction (90.166 ± 2.321 mg GAE/g) and n-butanol fraction (70.833 ± 1.699 mg GAE/g). However, low amount were detected in aqueous fraction (23.833 ± 3.171 mg GAE/g). The hydromethanolic crude extract (48.733 ± 0.865 mg GAE/g) are not the richest in total polyphenols compared to the other fractions. We found high amount of polyphenols in both polar (aqueous methanol and n-butanol) and non-polar (petroleum ether and chloroform) solvents. However, the difference were not found to be statistically significant for all the solvents.

3.3.2. Total flavonoids

Among extracts, maximum amount of flavonoid content was found in ethyl acetate and chloroform fractions (25.160 ± 0.016 and 22.023 ± 0.660 mg CE/g respectively) followed by aqueous fraction (14.995± 0.960 mg CE/g), n-butanol fraction (12.615 ± 0.884 mg CE/g) and the crude methanolic extract (10.726 ± 0.561 mg CE/g). The lowest amount of flavonoids was in petroleum ether fraction (8.387 ± 0.397 mg CE/g). The difference in amount of flavonoids were also, observed to be statistically insignificant.

Table 2: The content in total polyphenols, total flavonoids and condensed tannins for different tested *Gleditsia triacanthos* extracts.

Extracts	***Total polyphenols*** *(mg GAE/g DM)*	***Total flavonoids*** *(mg CE/g DM)*	***Condensed tannins*** *(mg TAE/g DM)*
CME	48.733 ± 0.865	10.726 ± 0.561	6.140 ± 0.148
PEF	66.800 ± 5.722	8.387 ± 0.397	3.410 ± 0.288
CF	131.667 ± 2.055	22.023 ± 0.660	0.305 ± 0.058
EAF	90.166 ± 2.321	25.160 ± 0.016	3.429 ±0.082
BF	70.833 ± 1.699	12.615 ±0.884	28.830 ± 0.269
AQF	23.833 ± 3.171	14.995± 0.960	0.567 ± 0.108

3.3.3. Condensed tannins

The results of the amounts of condensed tannins indicate that these molecules were not the majority constituents of the phenolic compounds of the different fractions, their quantities varied significantly ($p<0.05$) between 0.305 ± 0.058 and 28.830 ± 0.269 mg EAT/g. The most important amounts were in the n-butanol fraction (28.830 ± 0.269 mg EAT/g) and the crude methanolic extract (6.140 ± 0.148 mg EAT/g). The other factions had low levels of condensed tannins (0.305 ± 0.058 to 3.429 ± 0.082 mg EAT/g).

3.4. In vitro antioxidant activity

3.4.1. DPPH radical scavenging activity

The antioxidant activity through free radical scavenging activity (DPPH) method of the different extracts from honey locust pods at 1.562 to 200 µg/ml concentrations was determined (Figure 2). The percentage DPPH scavenging activities of all the extracts were dose dependent. A higher percentage inhibition means better antioxidant activity. All the extracts exhibited varying degrees of scavenging capacity.

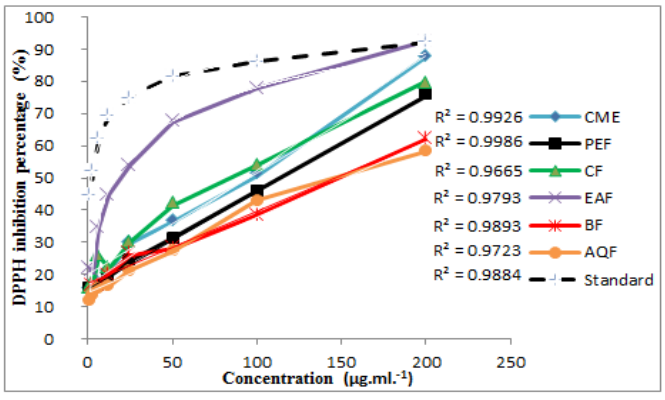


Figure 2: DPPH radical scavenging activity of *Gleditsia triacanthos* L pods extracts.

Since the IC_{50} value is inversely proportional to percent

inhibition, a lower IC_{50} value corresponds with a higher antioxidant power. The IC_{50} values for DPPH assay of the extracts have been given in Figure 3. A significant difference was observed ($P<0.05$) between the various solvents. The antioxidant activity of different extracts as equivalent to DPPH was in the order of: EAF > CME > CF > PEF> BF > AQF. The highest radical scavenging activity was obtained with the ethyl acetate fraction, with 92,436% inhibition of the free radical of DPPH at the concentration of 200 µg/ml, corresponding to the lowest value of IC_{50} (16,288 ± 0,299 µg/ml).

According to the obtained results, the crude methanolic extract and the chloroform fraction had a moderate antioxidant capacity; their IC_{50} were 93,964 ± 1,350 and 95,583 ± 3,529 µg/ml respectively, which is much higher than that of ascorbic acid whose value is of the order of 2,180 ± 0,0188 µg/ml. The petroleum ether fraction (114,619 ± 0,882 µg/ml), the n-butanol fraction (147,302 ± 3,320 µg/ml), and the aqueous fraction (152,465 ± 6,262 µg/ml), showed weak antiradical powers compared to the other fractions and the standard. From the results, it appears that the crude methanolic extract and his derivate fractions possess hydrogen-donating capabilities and can act as antioxidants.

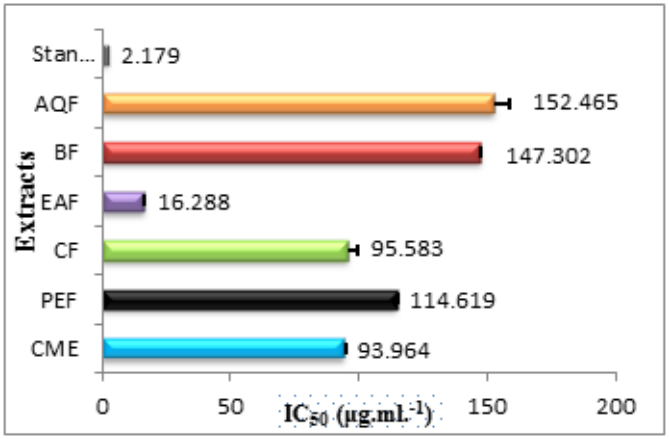


Figure 3: The IC_{50} values of DPPH scavenging effect of *Gleditsia triacanthos* L pods extracts

3.4.2. Total antioxidant capacity

Total antioxidant capacity of *Gleditsia triacanthos* L pods extracts using the phosphomolybdate method are displayed in Figure 4. A high absorbance value of the sample indicates its strong antioxidant capacity due to the intensity of the formation of the green phosphomolybdenum complex. Significant difference ($p < 0.05$) in total antioxidant capacity was observed between the different solvents.

In the ranking of the antioxidant capacity obtained by this method, the chloroform fraction showed higher phosphomolybdenum reduction (750,584 ± 129,793 AAE/g), followed by ethyl acetate fraction (362,688 ± 30,131 AAE/g). The values of total antioxidant capacities were also remarkably good for of the petroleum ether fraction (132,923 ± 14,474 AAE/g) and the crude methanolic extract (102,180 ± 3,728 AAE/g). These strong activities (TAC) can be attributed to the important levels of phenolic compounds in these fractions. The lowest antioxidant capacity was observed for the n-butanol and aqueous fractions (40,828 ± 2,766 and 10,723 ± 0,658 AAE/g respectively).

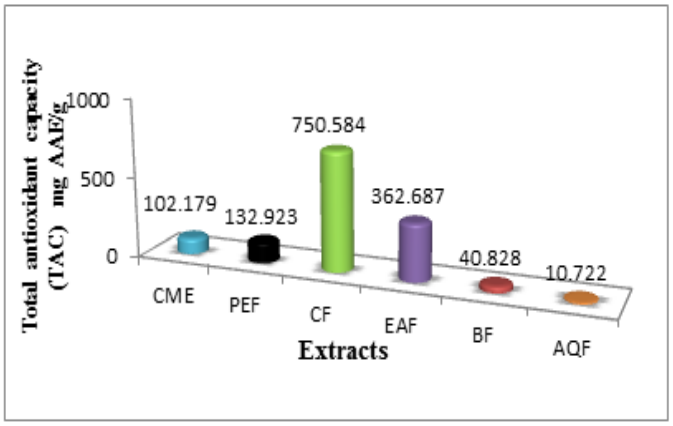


Figure 4: Total antioxidant capacity of *Gleditsia triacanthos* pods extracts

4. DISCUSSION

Extraction of active compounds from plant material raises currently a lot of interest because of their biological proprieties. Phenolic compounds are considered important natural antioxidants and represent one of the most abundant compounds in plants. They display several functions such as pigmentation, protection against ultraviolet rays, allelopathic action, defense against microbial attack and predators [19].

Biologically active compounds usually occur in low concentration in plants. An extraction technique is that which is able to obtain extracts with high yield and with minimal changes to the functional properties of the extract required [20]. Extract yield of *Gleditsia triacanthos* L pods prepared by maceration using increasing polarity solvents showed that the polar extracts (crude methanolic extract, n-butanol and aqueous fractions), were found to contain the highest yields. This result was in full agreements with other studies [7,19, 21, 22]. The yield is a relative parameter and depends on the plant species studied, the part used in the extraction, the drying and storage conditions, the content of each species in secondary metabolites and the nature of the solvent used in the plant extraction as well as the extraction method itself.

The phytochemical screening of the crude methanolic extract and his fractions from *Gleditsia triacanthos* L pods, revealed that tannins, alkaloids and cardiotonic glycosides were present in all the solvents used in this study, while anthraquinones were absent in all of them. The screening of chloroform, ethyl acetate and aqueous fractions showed the absence of sterols and terpenes. The petroleum ether fraction had the poorest phytochemical composition. Phytochemicals were distributed differently depending on the extraction solvent. Therefore solvent or the extraction agents used in the preparation of phytochemicals compounds must be suitable for dissolving the bioactive constituents and thus for separating them from the substances containing the compounds which are to be

extracted [23]. These phytochemical compounds, identified in the pods extracts may be responsible for the biological activity shown by *Gleditsia triacanthos* and the reason for their use in folk medicine for pain relief [24].

Few studies concerning the phenolic compound content of the studied specie were carried out. Therefore, and based on the phytochemical screening results, the total polyphenols, total flavonoids and condensed tannins contents of the different extracts of *Gleditsia triacanthos* L pods were estimated.

The values of the phenolic content clearly show that almost all the phenolic compounds present in the crude extract were depleted by organic solvents with increasing polarity and were concentrated in the chloroform and ethyl acetate fractions. This is confirmed by the relatively low levels of polyphénols observed in the aqueous fractions. The chloroform fraction showed the highest level of polyphenols. The results of El-Sayed et al.[7] on the leaves extracts of *Gleditsia triacanthos*, indicate that polyphenols content were 240.32 ± 1.56 mg GAE/g DM in the crude hydromethanolic extract, 160 ± 1.65 mg GAE / g DM in chloroform fraction and 275.42 ± 1.53 mg GAE /g DM in ethyl acetate fraction. These levels are very important compared to our results.

The total flavonoid content was quantified by the aluminum chloride method. The result shows that this species is rich in flavonoids mainly in the ethyl acetate fraction (25.160± 0.016 EC/g). This result is in full agreement with several studies, which proved that ethyl acetate solvent was the most efficient to retrieve flavonoids[7]. The flavonoids of this plant have very heterogeneous degrees of solubility resulting from their structural differences. Previous works [7, 25-28], have demonstrated that this botanical genus contains both types of flavonoids namely: the glycosylated flavonoids (vicenin, lucenine, isoorientin, orientine, vitexin and isovitexin), mainly present in Fabaceae, and aglycones flavonoids (luteolin and apigenin), which explains their high levels in solvents with different polarity. Less polar solvents are useful for extracting aglycone flavonoids, while more polar solvents such as water, ethyl acetate and alcohols are used to extract glycosidic flavonoids[29, 30].

The condensed tannins in our study are distributed with varying contents depending on the nature of the solvents. This inequality can be attributed to the difference in the degree of polymerization of the tannins extracted by the different solvents. Condensed tannins with low degrees of polymerization are soluble in water and polar solvents, while tannins with high degrees of polymerization are soluble in the alkaline solution[31]. Their amounts were higher in the n-butanol fraction and in the crude methanolic extract. However, our rates were much lower, than those, of earlier studies [32-35], indicating higher amounts of condensed tannins in *Gleditsia triacanthos* L pods, compared to our results.

Our results were in full agreement with the previous studies, which reported that the percent of the phenolic compounds from the plants depends on the nature of the solvent used in the extraction process of the plant. This can be explained by the differential solubility of the biomolecules extracted from the plant material and the selectivity of the solvents used. A polar solvent isolates the polar compounds and the nonpolar solvent extracts the nonpolar compounds, thus different solvents will give different extract compositions [36].

The antioxidant potential of the extracts was investigated by two in vitro assays, namely, DPPH and the phosphomolybdenum method (TAC). DPPH antioxidant assay is based on the ability of a potential antioxidant to scavenge the stable radical of 2,2-diphenyl-1-picrylhydrazyl (DPPH), a stable free radical contains an odd electron, which is responsible for a strong absorption band in the range of 515-520 nm. In the presence of antioxidant compounds, DPPH can accept an electron or a hydrogen atom from the antioxidant scavenger molecule, to be converted to a more stable DPPH molecule. As the reduced form of DPPH is pale yellow, the greater the free radical scavenging capacity of an antioxidant compounds, is identified by the more reduction of DPPH and the less purple color there is in the sample [37]. Our result clearly indicated that the ethyl acetate fraction presented an inhibition percentage of quite higher than other fractions (92,436 %) which was in agreement with the results reported in the literature [7, 22, 38, 39], indicating better DPPH free radical scavenging activity in the ethyl acetate solvent. This good antioxidant activity might be attributed to the presence of high amounts of phenolic compounds in this fraction. We suggest that the phytochemicals such as flavonoids, mostly present in the ethyl acetate fraction of *Gleditsia triacanthos* L pods could donate hydrogen to free radicals. In fact, these bioactive components could form complex compounds with the aluminum ion used in the flavonoid assay. The effectiveness of flavonoids as radical scavengers and metal chelators has been assigned to the hydroxyl group of the ortho position in the catechol structure (ring B), to the C2-3 double bond, conjugated to the C4 carbonyl group (cycle C) and the hydroxyl group at C5 (ring A) [40, 41]. The antioxidant potential can, also be related to the quality and structure of phenolic compounds than their amount in plant tissues.

The total antioxidant capacity (TAC) is a spectrophotometric assay based on the reduction of Mo (VI) to Mo (V) by the action of an antioxidant substance with the subsequent formation of a green phosphate/ Mo (V) complex with a maximum absorption at 695 nm [18]. The total antioxidant capacity of the extracts were higher in chloroform fraction (750,584 ± 129,793 mg AAE/g) followed by ethyl acetate fraction (362,688 ± 30,131 mg AAE/g). In addition, they also were the ones that showed the highest total polyphenolic and flavonoids contents among the studied extracts. These results suggest the importance of phenolic compounds on the antioxidant activity of these plant extracts, however we cannot neglect the potential influence of other bioactive compounds that may be extracted. Our results were different to those of El Sayed et al. [7]. According these authors, the leaves of *Gleditsia triacanthos* L had the highest total antioxidant capacities on the ethyl acetate fraction, (418.53 ± 3.78 mg AAE/ g),

followed by the crude methanolic extract (288.51 ± 2.19 mg AAE/g) and finally on the chloroform fraction (174.51 ± 2.92 mg AAE/g). The variable results of total antioxidant capacity of *Gleditsia triacanthos* L crude extract and factions may be explained by the fact that the transfer of electrons/hydrogen from antioxidants depends on the structure of the antioxidants. In the two methods, aqueous faction exhibited the lowest antioxidant activity than that of other extracts probably due to the low level of polyphénols observed in this fraction.

A significant difference was observed for the antioxidant activity in relation to different extraction solvents used ($p<0.05$), thus prompting the need for utilization of appropriate solvent for isolation of maximum amount of antioxidants. Indeed, several studies have reported variations in the biological activities of extracts using different solvents extraction. Iloki-Assanga et al. [42] confirmed that antioxidant activity of extracts is strongly dependent on the solvent due to the different antioxidant potentials of compounds with different polarity.

5. CONCLUSION

The present study demonstrate the richness in phenolic compounds as well as the large antioxidant capacity of the crude methanolic extract and fractions of *Gleditsia triacanthos* L pods. Phytochemical screening showed that the antioxidant activity of the extracts depend on the presence of phytochemicals such as, steroids, flavonoids, saponins tannins and alkaloids. Those phytochemicals were present diversely in different solvents. The choice of the extraction solvent is therefore essential because the secondary metabolites of the plant are distributed differently according to their respective polarity in each fraction, thus conferring on them medicinal and therapeutic properties. The study of antioxidant activity by two methods demonstrates that the ethyl acetate and chloroform fractions of *Gleditsia triacanthos* L pods could serve as potential sources of new antioxidant products. Further research is needed for the extraction and identification of active compounds present in these fractions, which will allow them to be used in pharmaceutical and human health fields. In vivo studies are also needed to understand the involvement of phenolic compounds in the antioxidant mechanism.

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